

# PATENT SPECIFICATION

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## (54) METHOD FOR THE PROPHYLAXIS AND TREATMENT OF DIARRHOEA IN DOGS

(71) We, NISSHIN FLOUR MILLING CO., LTD., a Japanese Body Corporate, of 19—12, Nihonbashi-Koami-Cho, Chuo-ku, Tokyo, Japan, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for the prophylaxis and treatment of diarrhoea in dogs. More particularly, this invention relates to a method for the prophylaxis or treatment of diarrhoea in dogs by oral administration of at least one bifidobacterium selected from *Bifidobacterium pseudolongum* and *Bifidobacterium adolescentis*, as isolated from the intestines and/or the faeces of dogs.

For the treatment of diarrhoea of dogs, it has been conventional to employ certain antibiotic substances which are commonly usable for the treatment of human diarrhoea. In such case, however, some pathogenic bacteria may be rendered antibiotic-resisting and such antibiotic-resisting bacteria are quite dangerous to the human body. Particularly for dogs which are kept indoors, a completely reliable therapeutic method for the prophylaxis and treatment of diarrhoea in dogs is essential, but there has not been found any satisfactory method.

As the result of our bacteriological studies on the diarrhoea in dogs, we have not recognized that such diarrhoea is closely related to the microorganism of *Bifidobacterium* genus which is one of the resident intestinal flora. Our inventive finding indicates that when the bifidobacteria are orally administered to dogs, then other harmful microorganisms which have been proliferated during the diarrhoea are unexpectedly reduced in their number to a normal level, with the number of the bifidobacteria being increased, so that the diarrhoea symptoms are appreciably reduced.

The present invention provides a method for the prophylaxis or treatment of diarrhoea in dogs, which comprises orally administering to dogs *Bifidobacterium pseudolongum* or/and *Bifidobacterium adolescentis* isolated from the intestines and/or faeces of dogs. The "*Bifidobacterium pseudolongum*" used herein is of the type b, and the "*Bifidobacterium adolescentis*" used herein is of the type b, c or d cf. Zentblatt für Bakt. I Org. 210, 52—64, especially page 56 (1969) these types being those isolated from dogs. The normally effective amount of such microorganism is at least  $10^6$  viable cells per day, preferably  $10^7$  or more viable cells per day for the purpose of prophylaxis and  $10^8$  or more viable cells per day for the purpose of therapy.

The above-specified bifidobacteria are usually resident in the digestive tracts of inherently healthy dogs, and do not show any oral toxicity. For instance, the freeze-dried organisms when orally administered have an LD<sub>50</sub> value of more than 20 grams per kilogram of the body weight.

The *Bifidobacterium*, i.e. either or both of the above-specified bifidobacteria, may be administered to the dogs in various forms. A vacuum-dried or liquid preparation is preferred. Examples of preferred forms which may be used are:—

(1) a preparation obtained by mixing the *Bifidobacterium* into a gelatinized starch paste, containing an amino acid, vacuum drying the mixture and then crushing it to granules. (2) a liquid preparation obtained by dispersing the *Bifidobacterium* in sterilized milk, and (3) a food composition obtained by mixing the *Bifidobacterium* with a dog food. Any dog food can be used, provided that

particular care is taken to protect the *Bifidobacterium* from attack by oxygen and/or heat.

The invention further provides a composition suitable for oral administration to dogs for the prophylaxis or treatment of diarrhoea in dogs, which composition comprises (1) *Bifidobacterium pseudolongum* and/or *Bifidobacterium adolescentis* isolated from the intestines and/or faeces of dogs, and (2) a dog food, sterilized milk or an amino acid.

Now the present invention will be illustrated by way of the following data.

Table I shows one embodiment in which the *Bifidobacterium* (*Bifidobacterium pseudolongum* type b, c or d, or *Bifidobacterium adolescentis* type b) is added to a dog suffering from diarrhoea at the administration amount of  $10^8$  viable cells per kg. body weight.

Table I.

	No. of the microorganism during diarrhoea (A)	No. of the microorganism after administration (B)	B/A
<i>B. adolescentis</i> type b, c, d	$7 \times 10^6$	$7 \times 10^8$	140
<i>B. pseudolongum</i> type b	$3 \times 10^5$	$2 \times 10^9$	667
<i>Streptococci</i>	$2 \times 10^9$	$1 \times 10^9$	0.5
<i>Enterobacteria</i>	$7 \times 10^8$	$3 \times 10^7$	0.04
<i>Clostridia</i>	$8 \times 10^8$	$6 \times 10^6$	0.08

As seen in Table I, the administration of the *Bifidobacterium* caused an increase in number of the administered organism while showing the appreciable decrease in number of other microorganisms especially proliferated during the diarrhoea.

Next, parasite-free dogs which were suffering from diarrhoea for at least three consecutive days were used as test animals, to which the *Bifidobacterium* preparation was administered with showing the result set forth in Table 2.

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Table 2.

Bacteria administered	Origin of said bacteria	Amount (Cell No./Head/Day)	No. of dogs tested	No. of scouring dogs after administration		
				1st day	3rd day	6th day
<i>B. pseudolongum</i> type b	dog	$10^6$	5	4	4	4
"	"	$10^7$	5	5	4	3
"	"	$10^8$	5	4	2	0
"	"	$10^9$	5	4	2	0
<i>B. thermophilum</i>	pig	$10^8$	5	5	4	4
<i>B. bifidum</i>	human	$10^8$	5	5	4	4
None	—	—	5	5	5	4
<i>B. adolescentis</i> type b	dog	$10^8$	5	5	1	0

As seen from the above, the bifidobacteria originating from other mammals than dog are not effective even at such a high amount as  $10^8$  or more viable cells.

Still next, newly weaned dogs of 30-days-old which were fed with milk and commercial dog food were used as test animals, which were administered with the *Bifidobacterium* preparation daily, commencing just after weaning, with the result set forth in Table 3.

Table 3.

Microorganism administered	Origin of said micro-organism	Amount (Cell No./Head/Day)	No. of dogs	No. of scouring dogs after administration		
				3rd day	7th day	14th day
<i>B. pseudolongum</i>	dog	$10^6$	4	3	2	2
"	"	$10^7$	4	1	0	0
"	"	$10^8$	4	0	1	0
<i>B. adolescentis</i>	"	$10^8$	4	1	0	0
<i>B. thermophilum</i>	pig	$10^8$	4	4	3	2
None	—	—	4	3	3	2

As seen from the above, the *Bifidobacterium* preparations originating from other mammals than dogs are not effective and it is best used in an amount of not less than  $10^6$  cells/day to ensure the intended effect.

## Example 1.

*Bifidobacterium pseudolongum* isolated from dog faeces according to a method described in "Zentralblatt fuer Bakteriologie, Infektionskrankheiten und Hygiene. 1 Orig. 195, p. 455—469 (1965)" was cultivated in a Briggs medium at  $37^\circ\text{C}$  for 20 hours under anaerobic conditions. The culture mass was centrifuged. The collected microorganism was dispersed at the ratio of 1:10 by volume with a

phosphate buffer solution (M/15) containing l-cysteine. The resulting dispersion was further uniformly dispersed at the ratio of 1:5 by volume with a viscous composition having the following formulation : 50 g. potato starch, 100 g. sodium glutamate or lysine hydrochloride, 19 g. l-cysteine, 15 g. gelatine and 500 ml. phosphate (M/15) buffer.

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The total mixture was added with wheat starch amounting to 70% of said mixture. The thus prepared pasty liquid was placed into a tray, and then dried at 37°C for 24 hours. The resulting sheet-like mass was crushed to granules.

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The granular preparation thus obtained was used for treatment of diarrhoea in dogs. The result is set forth in Table 4.

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Table 4.

Case No.	Sex	Age (year)	Body weight (kg)	Amount (viable cell (No./g	Prognosis				Evaluation
					1st day	3rd day	5th day	7th day	
1	M	4	32	$10^9$	+++	+	-	-	Effective
2	M	3	13	$10^9$	+++	+	+	-	"
3	M	0.5	20	$10^9$	++	+	+	+	"
4	F	8	12.5	$10^9$	++	+	++	++	Ineffective
5	M	0.7	12	$10^8$	+++	+	-	-	Effective
6	F	4	8.5	$10^8$	++	+	+	-	"
7	M	1	10	$10^9$	+++	++	-	-	"
8	M	0.5	3.2	$10^8$	+++	+	-	-	"
9	M	1.9	9.5	$10^9$	+++	++	+	+++	Ineffective
10	F	5	18	$10^9$	++	+	-	-	Effective
11	M	4	32	$10^9$	+++	++	++	+++	Ineffective
12	M	12	8	$10^8$	+++	++	+	-	Effective
13	F	2.5	40	$10^9$	++	+	+	-	"
14	F	3.5	30	$10^9$	++	-	-	-	"
15	M	2.8	3	$10^8$	+++	++	+	-	"

(Note)

+++ : Watery faeces (heavy diarrhoea).

++ : Loose faeces

+ : Soft faeces

- : Normal faeces (healthy)

## Example 2.

*Bifidobacterium pseudolongum* and *Bifidobacterium adolescentis* individually isolated from dog faeces were cultivated in separate Briggs media at 37°C for 20 hours under anaerobic conditions. The cultured broths were centrifuged individually. The collected microorganisms (*B. pseudolongum* and *B. adolescentis*) were suspended in sterilized milk so as to give a liquid preparation containing 10<sup>7</sup> cells of each of *B. pseudolongum* and *B. adolescentis* per millilitre of said liquid preparation.

This preparation was administered to newly weaned dogs at the dosage of 30 to 50 ml. per day for two weeks commencing just after weaning.

The group of the thus treated dogs showed only 10% occurrence of diarrhoea on average over 10 days after weaning, while the control group of non-treated dogs showed 75% occurrence of diarrhoea.

## WHAT WE CLAIM IS:—

1. A method for the prophylaxis or treatment of diarrhoea in dogs, which comprises orally administering to dogs *Bifidobacterium pseudolongum* or/and *Bifidobacterium adolescentis* isolated from the intestines and/or faeces of dogs.

2. A method as claimed in claim 1, wherein the *Bifidobacterium* is administered to dogs as a preparation obtained by mixing the *Bifidobacterium* into a gelatinized starch paste, containing an amino acid, vacuum drying the mixture and then crushing it to granules.

3. A method as claimed in claim 1, wherein the *Bifidobacterium* is administered to dogs as a liquid preparation obtained by dispersing the bifidobacterium in sterilized milk.

4. A method as claimed in claim 1, wherein the *Bifidobacterium* is administered to dogs as a food composition obtained by mixing the *Bifidobacterium* with a dog food.

5. A method as claimed in any preceding claim wherein a daily dose of at least 10<sup>6</sup> viable cells of the *Bifidobacterium* is administered.

6. A method for the prophylaxis or treatment of diarrhoea in dogs, substantially as hereinbefore described with reference to Example 1 or 2.

7. A composition suitable for oral administration to dogs for the prophylaxis or treatment of diarrhoea in dogs, which composition comprises (1) *Bifidobacterium pseudolongum* and/or *Bifidobacterium adolescentis* isolated from the intestines and/or faeces of dogs, (2) a dog food, sterilized milk or an amino acid.

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